UNIVERSITY OF PENNSYLVANIA

AN EXPERIMENTAL STUDY OF THE GROWTH EFFECTS OF THE ANTERIOR LOBE OF THE HYPOPHYSIS ON THE TEETH AND OTHER TISSUES AND ORGANS

WILLIAM GERSHOM DOWNS, JR.

A THESIS

IN MEDICAL SCIENCES

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

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Reprint from
The Journal of Dental Research, Vol. x, No. 5

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AN EXPERIMENTAL STUDY OF THE GROWTH EFFECTS OF THE ANTERIOR LOBE OF THE HYPOPHYSIS ON THE TEETH AND OTHER TISSUES AND ORGANS[1]

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I. INTRODUCTION[2]

Clinical literature abounds with references to variations in the dentition and to dental anomalies as the result of pituitary changes. Among such references are those of Kaplan (95), Howard (86), Graves (68), Kranz (101), Petzel (136), Ward (192), Cedercreutz (23), Englebach and Tierney (44), Stener (171), and others, all of them accepting in varying degrees the hypothesis that such a direct connection exists. In many cases this has been reported as sufficiently clear-cut to be pathognomonic of pituitary dyscrasias. A diligent search of the literature fails to reveal experimental studies of a careful nature pointing in this direction, although such a relationship has been experimentally demonstrated as existing between the parathyroids and the dentition. Among these studies in particular are those of Erdheim (45,46), Gies (63,64,65), Toyofuku (186), Hammett (71), and Toverud

[1] Presented to the Graduate School of the University of Pennsylvania in partial fulfillment of the requirements for the Degree of Ph.D. in Medical Science, June, 1930.

[2] The earlier portions of the experimental work involved here were done in the laboratories of the Department of Anatomy of the University of Pennsylvania. The director of this department, Dr. Eliot R. Clark, was Chairman of the Committee to which this thesis was presented.

(185), in all of which adequate evidence is presented that the texture, tensile strength, and the tissues surrounding the teeth respond to parathyroid change. In addition to these, many studies including those of Blum and Mellion (18), Cohen and Mendel (26), Höjer and Westin (79), Westin (196, 197), Howe (84, 85), Jackson (88, 89), Jones and Simonton (93), Karshan (96), Marshall (112, 113), McCollum, Simmonds, Kinney and Grieves (109), Mellanby (117, 118, 119), Meyer and McCormick (121, 122), Robb, Medes, McClendon, Graham, and Murphy (148), Erma Smith (160), Wolbach and Howe (201–204), Zilva and Wells (205), and Toverud (186), to name only a few of a very considerable number, have been made upon the effects of diet on the development and later pathology of the teeth and investing structures. Further than this many studies have been made upon the relationship of the growth hormones of the anterior lobe to metabolism. By consolidating the results of these various types of studies it becomes rather apparent that the hypophysis directly affects metabolism, and that metabolic changes are very apt to be reflected in the dentition.[3] My own earlier studies (35–38) gave sufficient reason for believing that there might be a connection between hypophysis and dentition, although not exercised in the direction of specific anomalies. Having in mind all of this background, pointing to the possibility of effects of the growth hormone in the anterior lobe on the development and pathology of the teeth and investing structures, the present study was begun.

In the experiments basic and preliminary to those herein described, a wide variety of methods was attempted and the results analyzed. Both homeo- and heterotransplants were utilized with about equal results, but were discarded because of the growing evidence, as presented by P. E. Smith, Engle (165, 167), Evans and Simpson (54), and others, that the anterior lobe elaborates at least two and possibly more hormones, only one of which apparently has a very direct bearing on the problem of growth. Various extracts containing the growth hormone were tried, but were discarded in favor of that described by Evans in his Harvey Lecture (47), and later (53, 54) somewhat modi-

[3] Studies in the direction of an effect of the hypophysis on "basal metabolism" have been largely negative. It is not felt, however, that O and CO_2 exchange necessarily constitute an indication of the fundamental metabolism of the organism.

fied by him and his associates. This extract gave the most consistently good results and, after an optimum dosage was obtained, seemed to have the fewest complicating features. Ablations and hypophysectomies were done on rats, mice and dogs, but were abandoned because of the very numerous complications involved—excepting those on dogs, in which the transbuccal complete hypophysectomy, as described by McLean (110) and somewhat modified to apply to younger and smaller animals, while attended by numerous difficulties, was found to be quite satisfactory and was utilized for the dog experiments.

The data in the ensuing study, upon which the curves for both normal and hypophysis-stimulated growth in mice are based, relate to controlled injection experiments begun in the fall of 1927. Since that time thirty-eight experiments have been conducted on animals from this stock at different seasonal periods and under somewhat varied environmental conditions, utilizing a total of nearly four thousand animals, over one half of which were treated in various ways by pituitary medication. The normal growth-curves for this stock, under a wide variety of conditions of handling, show remarkably little variation; and the later injection-stimulated curves, where a similar dosage (the optimum one) was utilized, likewise show little variation. Thus while quite numerous earlier experiments have laid the ground-work for and pointed the direction to the present enquiry, these consist of two complete series of studies: one, controlled injection experiments on mice; the other, hypophysectomy and injection experiments upon dogs. It was considered wise to utilize the two types of animals because of the fact that the one, mouse, permitted studies upon large numbers of animals throughout their entire lifetimes, as well as upon a continuously growing dentition and the cell changes and the chemical reactions at different periods; while the other, dog, permitted the simulation of both hypo- and hyper-secretions, and cell studies of larger structures and on a form having both deciduous and permanent dentitions not too far removed from the human type. For purposes of clarity, references to and comparisons with the literature have not been used in the parts of the paper (II and III) dealing with the experimental work proper, but have been reserved for a separate section (IV).

It is a pleasure to acknowledge my very great indebtedness to a

number of persons who have in large part made this work possible, particularly to Miss Katherine Cary and Miss Lucille Reed for assistance in the chemical analyses; Miss Anna E. Wilson and Miss Angelina Collins for secretarial assistance, and Drs. J. P. Peters, Raymond G. Hussey, H. S. Burr, Daniel C. Darrow, and Sophie B. de Aberle for aid in interpreting findings; Drs. E. M. K. Geiling, E. A. Sharp and C. C. Little, for assistance in obtaining materials; and Dr. W. S. German for assistance and guidance in the surgical technic involved; as well as the members of my graduate committee, whose courtesy and forbearance is truly unforgettable. To many others, to whom I am directly and indirectly indebted, I can only express my thanks in a general way, the list being too great to enumerate individually.

II. STUDIES ON MICE

The animals used in the following series of experiments were from the stock of "dilute browns," in Dr. C. C. Little's colony for the Department of Mammalian Genetics of the University of Michigan, at Ann Arbor. Since having been taken from that colony they were very closely inbred—for the most part, brother-to-sister matings. For these studies, the animals were separated into male and female groups, in order to avoid the variations due to breeding. When used in the tests, those of given litters were distributed as nearly as possible equally into the different groups. For this particular study, 192 animals were used—96 males, 96 females. All were twenty-one days old at the beginning of the experiment. It may be readily seen that, in order to obtain such a number, it had been necessary to plan a considerable time ahead, and mate a large number of animals at the same time in order to have offspring of the correct age and number.

The groups of 96 of each sex were divided into sub-groups of 48. One of these sub-groups in each case was to be treated, the other utilized as untreated control animals. Previous studies had demonstrated that there was no advantage in injecting the control animals, either with some other type of extract, such as that prepared from liver, or with normal saline. Using the controls as purely untreated animals simplified the procedure. At the initiation of the studies, each treated animal received 0.05 cc. of the alkaline growth extract of

the anterior lobe twice daily at equal intervals.[4] Otherwise all of these animals were handled in a similar manner; their diet was uniform, and the cleaning and changing of cages, and the other environmental conditions, were made as nearly identical as possible. When the animals had reached the age of six weeks, the dosage was increased to 0.1 cc.

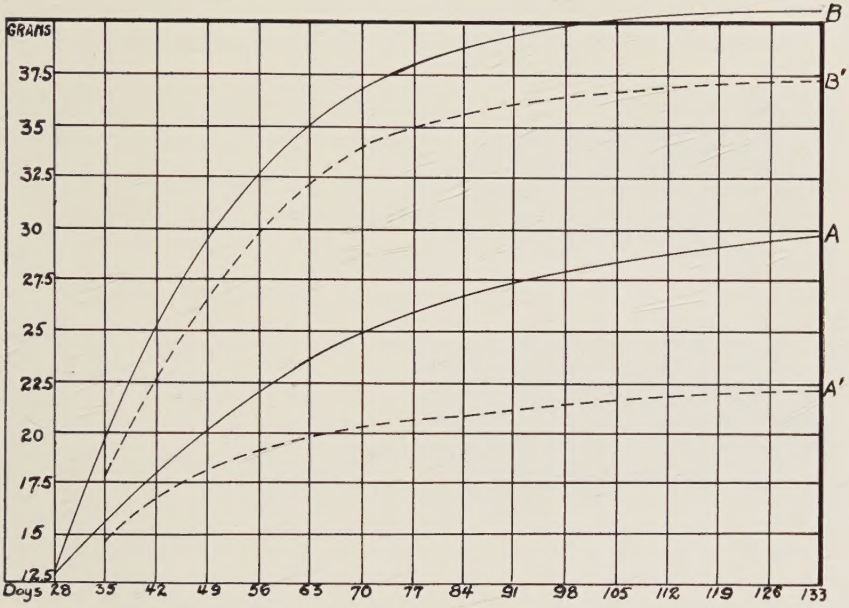

FIG. 1. GRAPH SHOWING GROWTH CURVES OF TREATED AND CONTROL MICE

A—Normal, untreated control animals. A^1—Same; data computed on a fat-free basis at all levels. *B*—Alkaline-injected animals. B^1—Same; data computed on a fat-free basis, after the method of Moulton.

twice daily, as earlier experiments had indicated that this was the optimum dosage. At the beginning of the experiments, two animals from each of the four sub-groups were killed and analyzed chemically. For four successive weeks this was done at weekly intervals, and then the interval was increased to two weeks. When animals were killed for

[4] This extract was prepared by Dr. E. M. K. Geiling of the Department of Pharmacology, Johns Hopkins Medical School, according to the formula outlined by H. M. Evans in his Harvey Lecture of 1923–1924, except that the pH was maintained at probably a slightly higher level than that of Dr. Evans' original extract. Our studies had indicated the desirability of this change.

analysis, all in the various sub-groups were weighed carefully and an average obtained for each sub-group, upon which is based the stimulated-growth curve shown in *fig. 1.* The associated normal-growth curve was obtained from average data for different periods and environmental conditions in a large number of studies, but it agrees so closely with the normal-growth curve for the series of control animals in this group of studies that they may be accepted as identical.

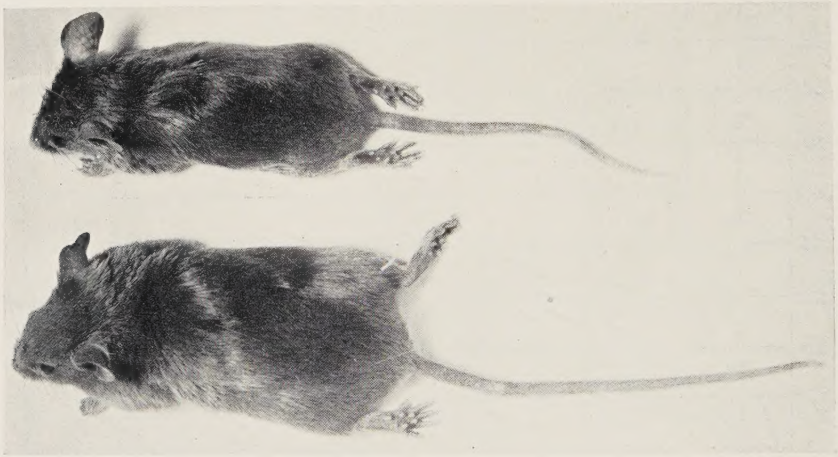

FIG. 2. TOP: UNTREATED CONTROL FEMALE MOUSE; TWELVE WEEKS OF AGE. BOTTOM: LITTER-MATE FEMALE; RECEIVED INJECTIONS OF THE ALKALINE PITUITARY EXTRACT TWICE DAILY

Chemical studies

As the animals were killed, those of a similar type of treatment and handling were frozen with "dry ice" until quite hard, and then ground separately in a food chopper with the finest cutter in place. This material was thoroughly mixed and ground again, and the process repeated six times until it was believed that the mass was thoroughly homogeneous. These thoroughly mixed masses of fresh whole-mouse tissue were then utilized for the chemical analyses. Approximately 5 gm. of each mass were taken for a determination of total nitrogen—Kjeldahl's were run on each in duplicate. Approximately the same amount was used for a determination of total fat content as indicated

below. The remainder[5] was dehydrated in an electric incubator at 110°C. for eight days and the loss of weight computed as water. This dried tissue was ashed in an electric muffle for twelve hours at a temperature of about 1400°F., and the percentage content of ash computed. The fat analyses were made by the method of Hertwig, Jamieson, Baughman, and Bailey (78), which is a modification of the Kerr-Sorber method. About 5 gm. of the material were put into a

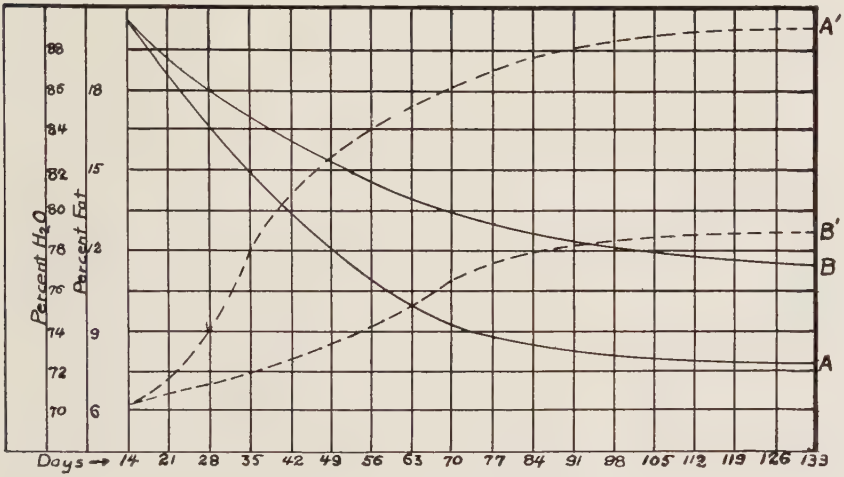

FIG. 3. GRAPH OF WATER AND FAT CONTENT OF FRESH WHOLE-MOUSE TISSUES AT ALL AGE LEVELS

A—Water content of tissues of control animals *A*[1]—Fat content of same tissues. *B*—Water content of tissues of injected mice. *B*[1]—Fat content of same tissues.

250 cc. Erlenmeyer flask, to which was added 60 cc. of alcohol and 6 cc. of KOH solution (100 gm. of KOH in 100 gm. of distilled water). A funnel was placed in the neck of the flask and the contents boiled gently on a steam bath for twenty minutes, or until saponification was complete. This mass after cooling was acidified with concentrated HCl and poured quantitatively into a separatory funnel. The fatty acids were shaken out with ether, using three successive portions of not less than 100 cc. each. The HCl in the ether solution was washed out

[5] Micro-chemical determinations of calcium and potassium, in the tissues of the last two series of animals, were made to ascertain whether the balance between these two had perhaps been altered. Such was not found to be the case.

with distilled water. The ether solution was considered free from HCl when the wash-water no longer showed a pink tint with methyl-orange. The ether solution of fatty acids was dehydrated over anhydrous sodium sulphate, centrifuged free from the salt, the ether evaporated, and the residue dried to a constant weight of 80°C.

This type of handling and computation was continued over a total period of sixteen weeks. The accompanying graphs (*figs. 3* and *4*) were prepared from the data so obtained. In the chemical analysis it was found that the tissues of the pair of animals from each sub-group, handled separately throughout as stated above, were so nearly the

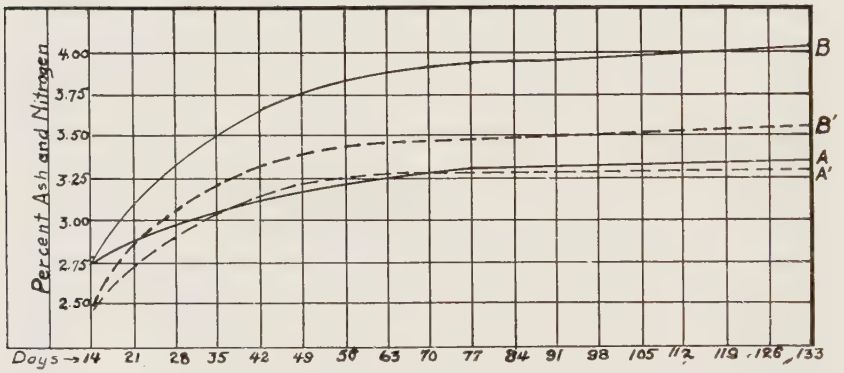

FIG. 4. GRAPH SHOWING TOTAL ASH AND TOTAL NITROGEN CONTENTS OF FRESH WHOLE-MOUSE TISSUES AT ALL AGE LEVELS

A—Total ash content of fresh whole-mouse tissue obtained from untreated control animals. A^1—Total nitrogen content of tissues of same animals. *B*—Total ash contents of tissue from alkaline-injected animals. B^1—Total nitrogen content of same tissues.

same, irrespective of sex, that the data were grouped simply into experimental and control series. In addition to the chemical studies outlined above, and at irregular periods extending over the entire experiment, animals representative of each experimental group were killed and autopsied, and sections made of the more important visceral organs and of the maxillae and teeth.

Findings. The accompanying graphs (*figs. 3* and *4*) show that the animals receiving the alkaline anterior-pituitary extract grew at a markedly more rapid rate, and to a considerably larger size, than the untreated control animals (*figs. 1* and *2*). This difference apparently

had no relationship to the sex of the animals, although with increasing age each sub-group gradually lost in water-content, which remained considerably higher at all levels in the alkaline-injected animals than in the uninjected controls at the same levels (*fig. 3*). The injected animals did not take up or show nearly as high a proportion of fat as the uninjected controls. Each type of animal, with advancing age, showed a tendency to increase gradually the total mineral content of the tissues. This was, however, considerably more evident in the injected animals than in the controls (*fig. 4*).

Moulton (127) has shown that in order to compare data such as these it is necessary to make the computations on a fat-free basis. For that reason the data were computed on this basis as well as on that of the whole-tissue. While this method naturally varied the percentages shown, the relationship between the contents of injected and control animals was not materially altered. As animals of each type aged, the total nitrogen in the tissue tended to increase. While this was apparently more true of the alkaline-injected animals than of the controls, the very slight difference was considered as being well within the margin of error for such computations.

Inasmuch as various methods of preparation of the growth-hormone extracts have been used by different workers, several of these were tried as a check. Thirty-four animals of an average weight of 12 gm. were divided into four nearly equal groups, and the sexes kept segregated. The animals in one group received injections of Evans alkaline extract as prepared for me by Dr. Geiling; one, the Teel "globulin" fraction, also prepared in Dr. Geiling's laboratory; and one the Parke-Davis product—0.1 cc. twice daily in each case. Each product resulted in a stimulation of growth, which was most for the Teel "globulin" fraction, and least for the Evans extract prepared by Dr. Geiling. There was a notable difference between the effects of the "globulin" fraction and the other two, but much less difference between the effects of the Evans extracts as prepared by Parke-Davis and by Dr. Geiling.

An effort was made to apply the method of Marshall by injecting trypan blue or naphthalene brilliant blue, or one of the alizarin dyes, to obtain definite lines of deposition of calcium in the teeth and thus show the rate of growth. However, the outcome was not satisfactory, and no conclusions may be drawn from this portion of the study.

But the method of marking the constantly growing incisors with a fine thin disk on a dental mount, and marking again at regular intervals, thus recording differences in rate of growth in these teeth, was fairly satisfactory. By this method it was found that the incisor teeth of injected animals showed a more rapid growth than those in control animals. In this series it was determined that the average rate of

TABLE 1

Data showing the growth rates of mouse incisors; expressed in millimeters

ANIMALS*	1ST WEEK	2ND WEEK	3RD WEEK	4TH WEEK	AVERAGES
Injected group					
47	1.2	1.2	1.4	1.3	1.275
51	1.3	1.4	1.4	1.4	1.375
52	1.3	1.3		1.3	1.3
54	1.3	1.2	1.3	1.3	1.275
61	1.4	1.4	1.4	1.4	1.4
63	1.3	1.3	1.4	1.3	1.375
92	1.3	1.2	1.3	1.2	1.25
93	1.4	1.3	1.4	1.4	1.375
				General average,	1.328
Control group					
101	1.1	1.1	1.0	1.1	1.075
103	1.0	1.0	1.0	0.9	0.975
107	1.2	1.1	1.1	1.1	1.125
116	1.1	1.0	0.9	1.0	1.0
122	1.0	1.0	1.0	1.0	1.0
123	0.9	0.9	0.9	1.0	0.975
133	0.9	1.0	0.8	0.9	0.9
134	1.0	0.9	1.0	0.9	0.95
				General average,	1.000

* Figures in the first column represent the identification numbers of the animals.

growth of the incisors was about 1.0 mm. per week in normal untreated animals. Of thirty-eight injected animals, each showed a greater rate, the average being 1.3 mm. per week. *Table 1* gives the actual measurements of one group of injected animals and one group of controls studied in this way for a period of four weeks, the data being typical of the growth rate throughout.

Microscopic Studies

The most marked cell difference in the two types of animals was noted in the livers and internal female genitalia. Somewhat less marked, but quite evident, were changes in the lymphogenic tissues.

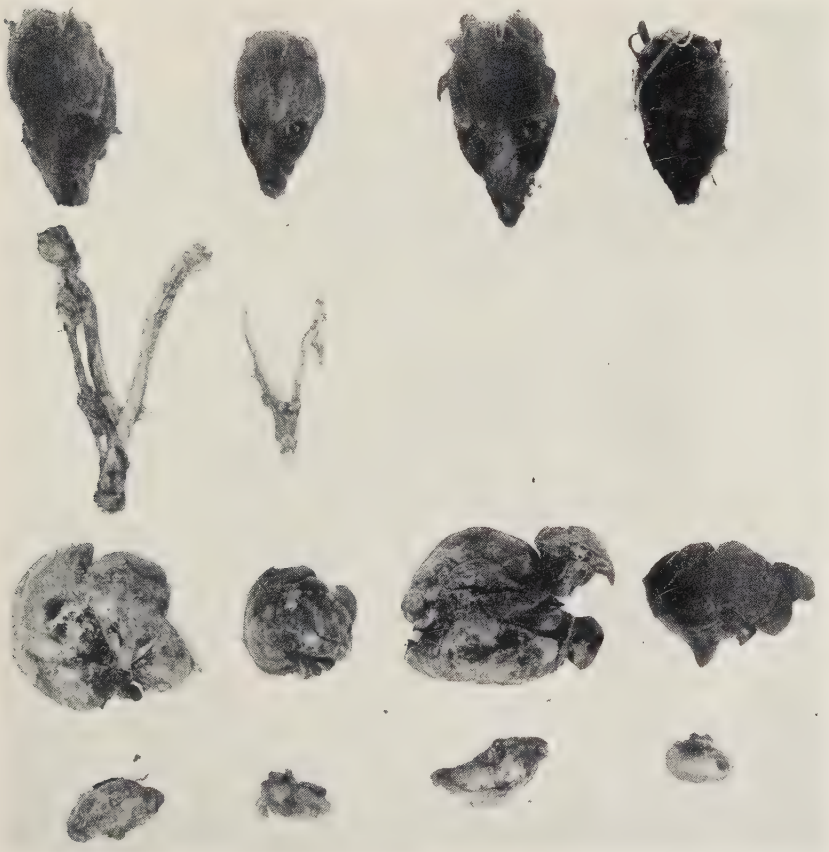

FIG. 5. WHOLE ORGANS FROM TREATED AND CONTROL LITTER-MATE MICE, TEN WEEKS OF AGE

Vertical rows, left to right. *First row:* downward—skull, internal female genitalia, liver, kidney, of injected female. *Second row:* same organs of untreated control litter-mate female. *Third row:* skull, liver, kidney, of injected male. *Fourth row:* same organs of untreated control litter-mate male. (Male genitalia show no distinctive difference.)

Less striking, but evident, were changes in the kidneys. Other variations were too slight to require attention. A very marked splanchnomegaly, in all of the injected animals, was most noticeable in the liver and internal female genitalia; less so in the kidney. In the liver, there was a very great increase in the size of the cell nuclei in the injected animals; a slight increase in the total size of the cells; marked destruction of the cells around the central vein, which extended half-

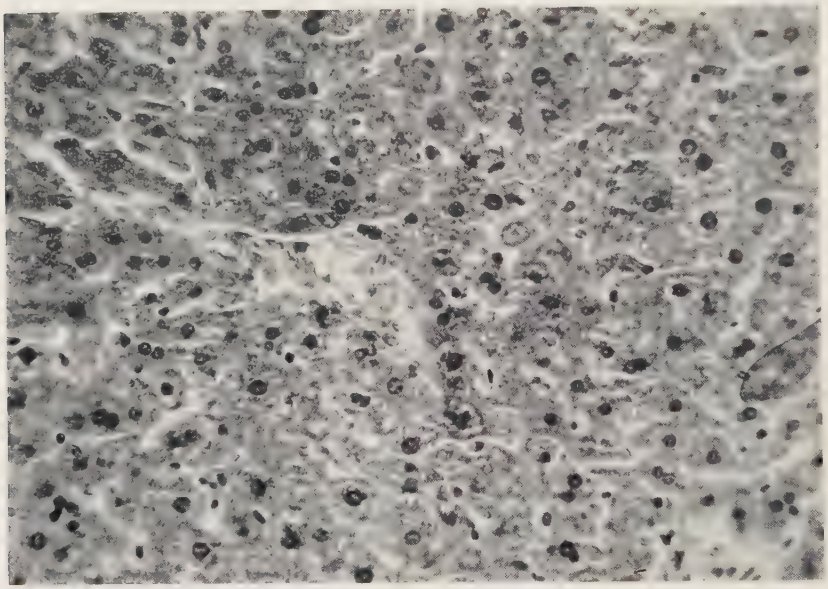

FIG. 6. PHOTOMICROGRAPH OF LIVER OF UNTREATED CONTROL FEMALE, SHOWING COMPACT NUCLEI AND GENERAL COMPACT ARRANGEMENT OF HEPATIC CELLS (× 160)

way or more through the lobules, in many cases amounting to complete destruction and necrosis of the cells; some increase in the lymphocytes in these regions; and possibly a slight tendency to increase in interstitial connective tissue; the blood vessels, in particular the central veins, were considerably enlarged and thin-walled (*figs. 6* and *7*).

Findings. In the uterus, tubes, and ovaries, besides increase in gross size (*fig. 5*), there was no apparent difference in the stage of development of the ovarian follicles; a possible slight advancement in the stage of the endometrium and endometrial glands; a thickening and

increase in quantity of the myometrium and walls of the tubes (the most marked change); and a possible increase in the epithelial lining of the uterus, and particularly of the vagina. In the kidneys most of the changes were in the region of the convoluted tubules, where there was much cloudy swelling in the tubular epithelium and some increase in the connective tissue elements between the tubules; also a notable congestion of the capillaries.

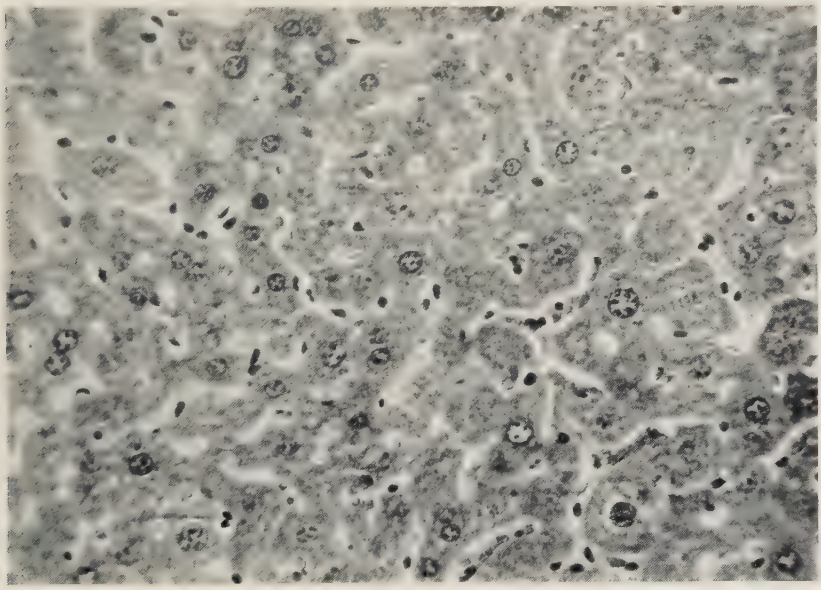

FIG. 7. PHOTOMICROGRAPH OF LIVER OF INJECTED FEMALE

Litter-mate of one in *Fig. 6*. Shows larger, less dense nuclei, larger hepatic cells, and distension of blood sinuses in general (× 160)

The mandibulae of many of the animals, injected and control, were fixed in formalin, formol-Zenker's, or Bouin and Susa fixatives; decalcified in 5 per cent nitric acid or 5 per cent trichloracetic acid; neutralized with lithium carbonate in 1 per cent solution; embedded in paraffin, or celloidon, or double-embedded; sectioned; and stained variously with methylene blue, iron hematoxylin, Delafield's hematoxylin and eosin, haemalum and Congo red, or Masson's trichromic stain. Of each series a few were tried with the mitochondrial stains of

Cowdry and Benda. The most careful examination showed no regular variation in the histology of the continuously growing incisors, molars, or surrounding bone. There was no evidence of osteodentin formation in the pulps, and no change in the odontoblasts or ameloblasts. The mitochondrial stains showed no regular variations in the cytoplasmic inclusions. Microscopic examination revealed no notable difference in density of bone, dentin, or enamel. In short the microscopic pic-

FIG. 8. ENLARGED PRINT OF X-RAYS OF MANDIBULAE OF EXPERIMENTAL AND CONTROL MICE

Top, left: mandible of injected female. Bottom, left: mandible of litter-mate control female. Top, right: mandible of injected male. Bottom, right: mandible of litter-mate control male, showing difference in both size and density between injected and control animals.

ture was entirely negative, so far as mandible and teeth were concerned (*figs. 25–31, 35–37*).

X-ray studies of the maxillae and bones of the two types of animals were made. Owing to the very small size of the long bones, the findings relating to them were not significant. The maxillae and the incisors, however, were much larger and denser in the injected animals than in the controls (*fig. 8*). Whether this is disproportionate to the total rate of growth of the animals is a matter for conjecture. Although

difficult to demonstrate, it is felt that the increase in rate of growth as well as the increase in size, of the continuously growing teeth of the injected animals, is only about proportional to the rate of increase of their general somatic growth, and represents no specific action.

III. STUDIES ON DOGS

Owing to the well known variations of individuals, even within fairly regular types of animals, it seemed essential in these studies to use litter-mates of a closely inbred type. To facilitate handling it was necessary that the animals should be small. Fox terriers in fairly large litters, and of a closely inbred strain running from eight to ten weeks of age, were selected. When the first litter was obtained, attempts were begun to hypophysectomize several of each sex. The method outlined by McLean (110) was in general utilized; but, owing to the fact that his animals were obviously larger, some changes were necessary. A modification of the operating table and appurtenances described by McLean was worked out in the animal operating room of the Department of Surgery of Yale University, which made it possible to hold the dogs immovable, and have access to the soft palate and pharynx with relative ease.

In the first few operations chloralose, paraldehyde, and amytal were tried variously to determine which would give the best results. These were obtained consistently with amytal when dogs, which had fasted for twenty-four hours, were given intraperitoneally 50 mg. per k. about three quarters of an hour before the operation, and their stomachs washed before receiving a small amount of atropin by stomach tube to lessen secretion from the mucous membranes on which the operation was to be done. The initial stages of the operation were done as outlined by McLean until the point of incising the muco-periosteum was reached. Here, with animals so small as those in use, it was not practicable to use guy sutures on the delicate tissues involved and in the small working space allowed. Instead, a V-shaped flap was cut and turned back posteriorly out of the field of operation. The bony fault was then made with a large round dental bur, and cautiously followed down to the cranial layer of periosteum, which was carefully denuded of all bony fragments, incised with a small lance-shaped instrument, and access made to the underlying dura. With practice

it was possible to locate the dura overlying the hypophysis and to incise that. When this was cautiously done and a small fault made, the hypophysis gradually worked through the incision as the pulsations in the intra-cranial pressure changed its position. After it had completely pushed through, it was carefully excised with a small round rongeur. In many instances, in completing these stages, openings were made into the circular sinus, which lies very close in the dog, whereupon the animals bled to death or died shortly after the opera-

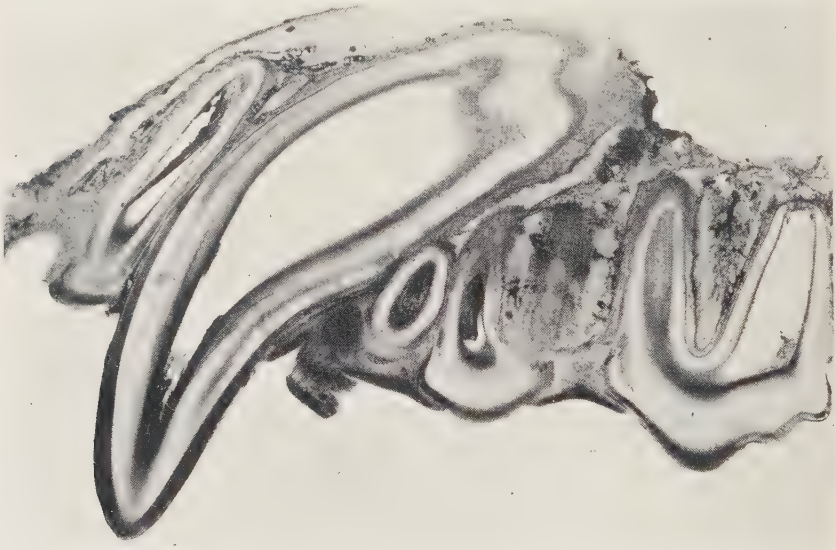

FIG. 9. ENLARGED (4 ×) GROUND SECTION THROUGH LOWER RIGHT CANINE OF INJECTED MALE DOG AT AUTOPSY

No lines of deposition of dye can be seen. All elements are approximately same size.

tion from subdural hemorrhage. This seemed to be the cause of the greatest loss. Following hypophysectomy, a few animals were lost from convulsions, which at autopsy seemed in no way connected with hemorrhage or local trauma. These losses were classed as being due to "acute pituitary insufficiency," although the term is considered quite unsatisfactory. Following removal of the entire gland in the earlier cases, an attempt was made to close the bony fault with bone wax or with a plug of bone, but the results were so unsatisfactory that

in the later operations the effort was discontinued. Instead, the area was carefully cleansed with 1 per cent mercurochrome and the flap in the muco-periosteum brought down over the flaw, where it stayed in place fairly well. The soft palate was sutured, and the area again scrubbed with mercurochrome; and the dog kept warm until well out of the anesthesia.

Four dogs in all, two of each sex, were finally available. Each was grouped with one of the same sex and litter that received the alkaline

FIG. 10. ENLARGED (4 ×) GROUND SECTION THROUGH LOWER RIGHT CANINE OF CONTROL MALE DOG, LITTER-MATE OF THAT REPRESENTED IN FIG. 9

No lines of deposition of dye can be seen. All elements are approximately same size.

extract (described by Evans and somewhat modified as indicated earlier); another of the same sex and litter was kept untreated, but handled and fed in an identical manner as a control.[6] As soon as it became evident that a given hypophysectomy had been done with reasonable

[6] Owing to the quantities of extract necessary for these studies, it became necessary to call for further help. Arrangements were made with the department of experimental medicine of Parke, Davis & Company to supply, in large quantities, the alkaline extract made in accord with Evans, Cornish, and Simpson's last modification (55).

hope of success, these groupings were made, and the animals weighed, and examined for variations in hairy coat, build, and dentition. They were re-examined and weighed at intervals of two weeks throughout the progress of the experiment. The animals that received the injections of alkaline extract of the anterior lobe, at the beginning were given intra-peritoneally 3 cc. daily. The injections were made with reasonably aseptic precautions, and the extracts warmed to approximately body temperature before injection. The dosage was gradually

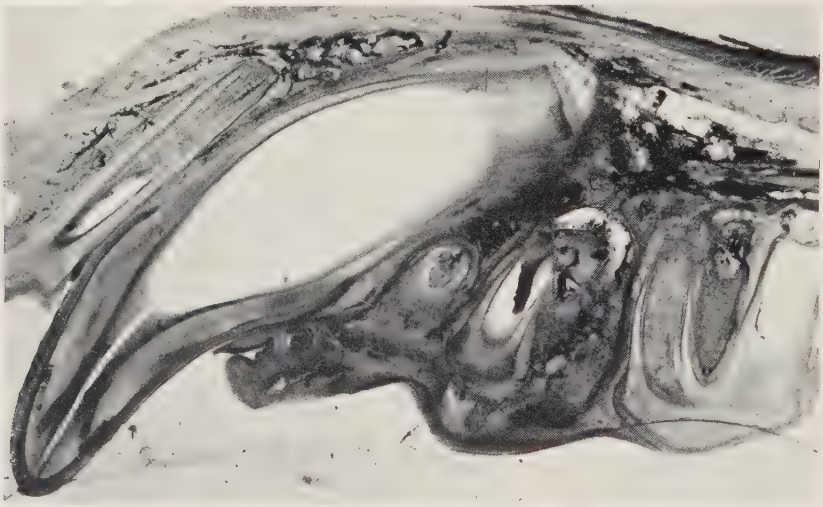

FIG. 11. ENLARGED (4 ×) GROUND SECTION THROUGH LOWER RIGHT CANINE OF HYPOPHYSECTOMIZED MALE DOG, LITTER-MATE OF ANIMALS REPRESENTED IN FIGS. 9 AND 10
No lines of deposition of dye can be seen. All elements are approximately same size

increased as the animals became older and larger. By varying the dosage and observing the response, the optimum results were obtained from a dosage of slightly more than 1 cc. per pound of body weight.

As has been said, the experiment was begun with four groups of animals: two, male; two, female. In about six weeks, one of the male groups was killed and autopsied. Thus early in the course of the study, there were noted some slight changes that accorded with results in the later autopsies. Fourteen weeks after the beginning of the experiment, a group of females was killed and carefully examined. Two days

FIG. 12. LITTER-MATE MALES AFTER SIX WEEKS OF TREATMENT

Left: hypophysectomized. *Center:* untreated control. *Right:* injected. At outset, the hypophysectomized animal was slightly larger than the control; the injected animal was much the smallest.

FIG. 13. THE SAME GROUP OF LITTER-MATE MALES, IN FIG. 12, AFTER FIVE MONTHS OF TREATMENT

Note the loose, baggy skin and general "cretinoid" appearance of the operated animal; also the foot, ankle, and postural changes in both animals; and the marked size-increase in the injected animal.

later, owing to a pulmonary infection, it was necessary to sacrifice the other group of females, which were thoroughly studied. Of the remaining group of males, after twenty-one weeks the injected animal died from an apparent toxicosis, which made it necessary then to autopsy this group.

General findings. The permanent dentition erupted at a much earlier age in the animals that received the alkaline extract of the anterior

TABLE 2

Summary of effects noted at autopsies (one group of dogs)

	HYPOPHYSECTOMIZED	CONTROL	INJECTED
Gross weight at start	5 lb., 14 oz.	5 lb., 2 oz.	4 lb., 2 oz.
Gross weight at end	8 lb., 2 oz.	8 lb., 10 oz.	10 lb., 2 oz.
Amount of fat present	Large amount	Little	None
Liver	Wt. 118.4 gm.; normal	Wt. 188.5 gm.; normal	Wt. 268.9 gm.; focal necrosis
Genitalia	Small, delicate	Moderate	Large, heavy
Tongue	Small, rough	Moderate	Large, heavy
Skull	Small; under-developed in frontal and pre-maxillary regions	Moderate; normal	Large; frontal region bulging; maxillae large and heavy
Mandible	7.5 cm. x 2 cm.; light in texture	8.0 cm. x 2.3 cm.; normal	8.5 cm. x 2.8 cm.; heavy, dense
Teeth	Delayed eruption; "tumbled" in arch	Normal	Advanced eruption; "spaced" in arch
Cuspids	Greatest diameter, 6.1 mm.	Greatest diameter, 6.0 mm.	Greatest diameter, 6.1 mm.
	Tip to apex, 22 mm.	Tip to apex, 22 mm.	Tip to apex, 21 mm.
	Thickness of wall, 2 mm.	Thickness of wall, 2 mm.	Thickness of wall, 2 mm.

lobe than in the controls or the hypophysectomized animals. The latter were much slower in this respect than the controls or the injected ones. There was no observable variation in the size or form of the teeth, but some increase in the size of the maxilla in the injected animals, and a decrease in these bones in the hypophysectomized ones, causing a definite spacing of the teeth of the injected dogs, and crowding

and "flaring" of the teeth in the hypophysectomized ones, neither condition occurring in the controls (*figs. 23* and *24*). The injected dogs had a somewhat denser growth of hair, and were sleeker in appearance and much larger for a considerable period of their growth, while the hypophysectomized animals showed the opposite tendency (*figs. 12* and *13*). Strangely, although the difference in bony development between the two groups was very noticeable, both of the treated types

FIG. 14. PHOTOGRAPHS, HALF-SIZE, OF OVARIES, TUBES AND UTERI OF TWO GROUPS OF FEMALE DOGS

4 and *9*, organs of hypophysectomized animals; *5* and *7*, organs of control animals; *6* and *8*, organs of injected animals.

had a weakness of the ankles that was not observed in the controls. The injected dogs showed an extreme lethargy, and dullness of a sort, while the hypophysectomized ones were very nervous and excitable. At autopsy, the injected animals in addition to marked general gain in weight, exhibited an even disproportionate increase in the size of the viscera, most marked in the liver—in the females, in the uterus and tubes; no similar changes were noticed in the internal male genitalia, but in both sexes, the kidney was somewhat larger. The hypophy-

sectomized animals showed the greatest amounts of subcutaneous, mediastinal, and mesenteric fat; the injected animals, the least. In the injected females, there was a marked tendency to enlargement of the external genitalia and vulvae, which were pendulous and hypertrophic; the hypophysectomized animals were definitely infantile in this direction, and showed an almost complete lack of development in the internal genitalia (*fig. 14*). In the injected animals the tongue was considerably increased in size, and there was general unwieldiness in its use; no change in this respect was noticed in the hypophysecto-

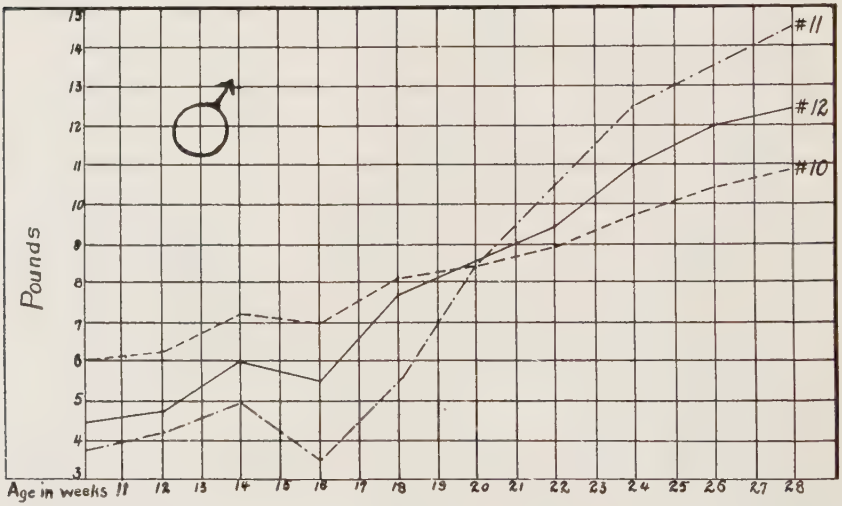

FIG. 15. GRAPH OF GROWTH CURVES FOR LITTER-MATE MALES SHOWN IN FIGS. 12 AND 13
11, alkaline-injected; *12*, untreated control; *10*, hypophysectomized.

mized dogs. The changes in a group of females are summarized in *Table 2*, and the autopsy protocols appended at the end of the paper. The protocols for the other group of females, while differing in details, reveal essentially the same relative findings as those in *Table 2*. The same conditions governed in a somewhat more advanced degree in the group of males that was continued six weeks longer.

In each group, the heaviest animal had been selected for hypophysectomy, the lightest for injections, as there was excellent reason to believe that the operation would retard the rate of growth, and the injections accelerate it. At the end of each experiment, the hypophy-

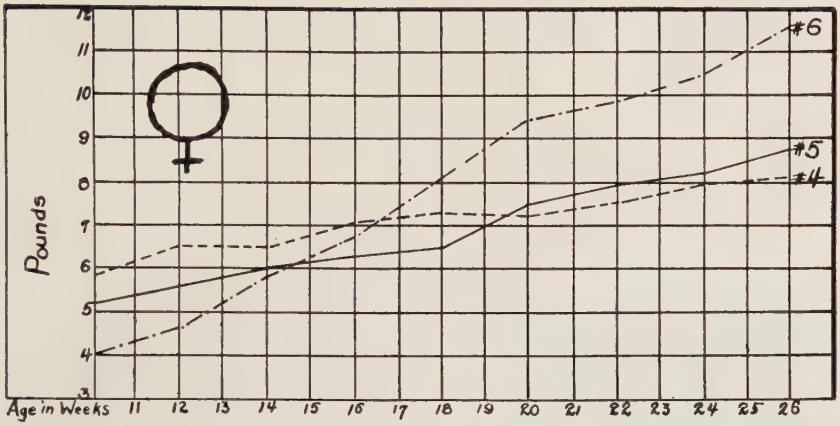

FIG. 16. GRAPH OF GROWTH CURVES FOR LITTER-MATE FEMALES AUTOPSIED AFTER SIXTEEN WEEKS OF TREATMENT

4, hypophysectomized; *5*, untreated control; *6*, alkaline-injected.

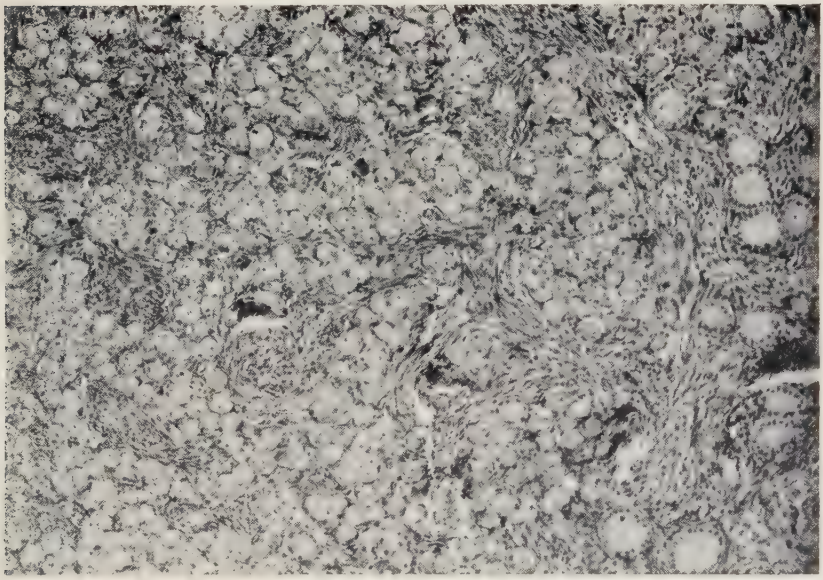

FIG. 17. PHOTOMICROGRAPH OF OVARY OF HYPOPHYSECTOMIZED DOG (LITTER-MATES FIGS. 18 AND 19). SHOWING INFANTILE TYPE OF OVA, WITH NO TENDENCY TO FOLLICLE FORMATION (× 140)

sectomized animals were the lightest; the injected, by far the heaviest (*figs. 15* and *16*). The differences noted in the viscera were constant for the animals on a similar type of handling.

Microscopic findings. Liver. In the liver there was a slight increase in the size of the cell nuclei in the injected animals; atrophy and necrosis of the cells at the center of the lobules; and some infiltration of plasma and polymorphonuclear cells as well as lymphocytes, and a slight increase in the interstitial connective tissue, in these re-

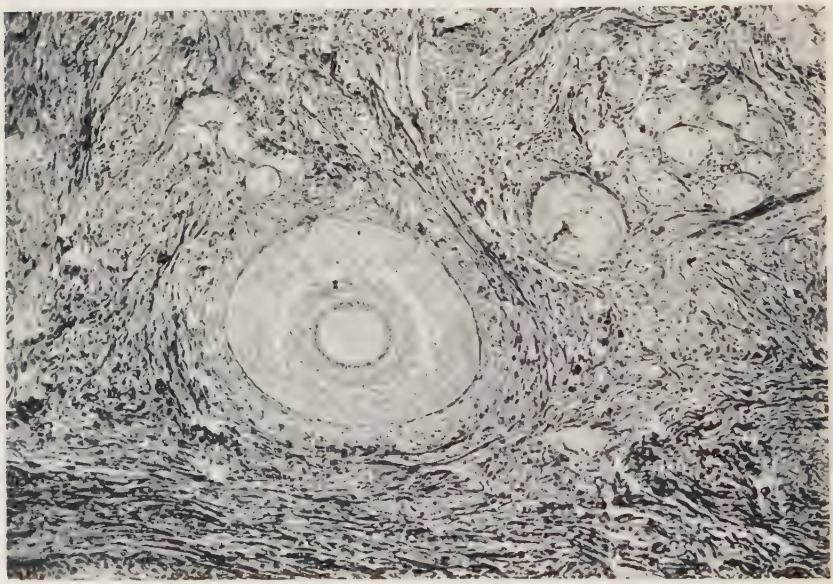

FIG. 18. PHOTOMICROGRAPH OF OVARY OF UNTREATED LITTER-MATE CONTROL (FIGS. 17 AND 19), SHOWING ADVANCED FOLLICLE FORMATION (× 140)

gions. There was also some congestion of the blood sinuses. The nuclei of the cells of the injected dogs take the nuclear stains less intensely than those from the control or hypophysectomized animals, the latter showing a very deep nuclear stain.

Kidney. The kidneys of the control and hypophysectomized animals showed no advanced microscopic differences. However, in the injected animals, there was a moderate degree of cloudy swelling in the tubular epithelium, most apparent in the convoluted portions. In

these loci also, there was infiltration of lymphocytes and increase in interstitial connective tissue, and a slight amount of congestion of the capillaries.

Ovaries. The ovaries of the hypophysectomized animals were very small and infantile, containing no advanced follicles and a very dense ovarian stroma (*figs. 17, 18,* and *19*). The tubes and uteri, grossly much smaller, exhibited no advanced stages of endometrial changes, and only a very thin wall and myometrium. In the controls the

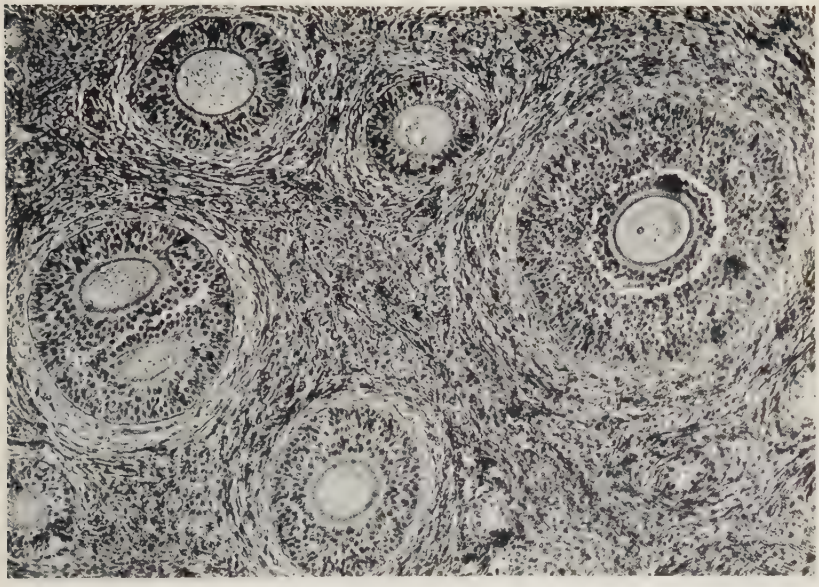

FIG. 19. PHOTOMICROGRAPH OF OVARY OF INJECTED LITTER-MATE (FIGS. 17 AND 18), SHOWING FOLLICLES AT A STATE OF ADVANCEMENT THAT DIFFERS PROBABLY ONLY A FEW HOURS FROM THE CONTROL, BUT HAVING A LARGER AMOUNT OF STROMA (× 140)

ovaries, tubes, and uteri were larger. The ovaries showed a number of advanced but not ripe follicles; the tubes and uteri, some changes in the lining glands of a proliferative character, and a very great thickening of the walls and myometrium. In the injected animals, compared with the controls, there was no change in the condition of the follicles or endometrial glands or epithelium, but a very marked increase in the stroma in the ovary, and in the myometrium and connective tissue in the tubes and uteri.

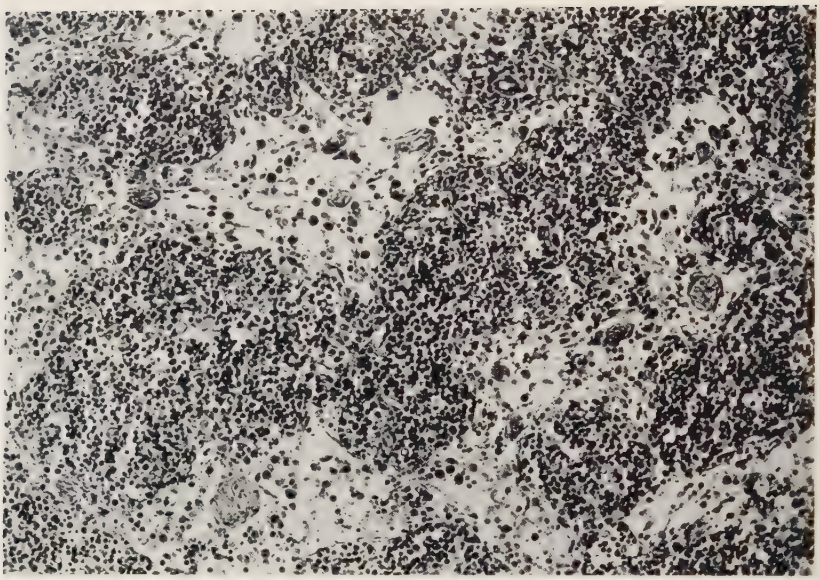

FIG. 20. PHOTOMICROGRAPH OF CERVICAL LYMPH-NODE FROM CAROTID GROUP IN HYPOPHYSECTOMIZED FEMALE (LITTER-MATES, FIGS. 21 AND 22), SHOWING RELATIVELY GREAT DENSITY OF LYMPHOCYTES (× 80)

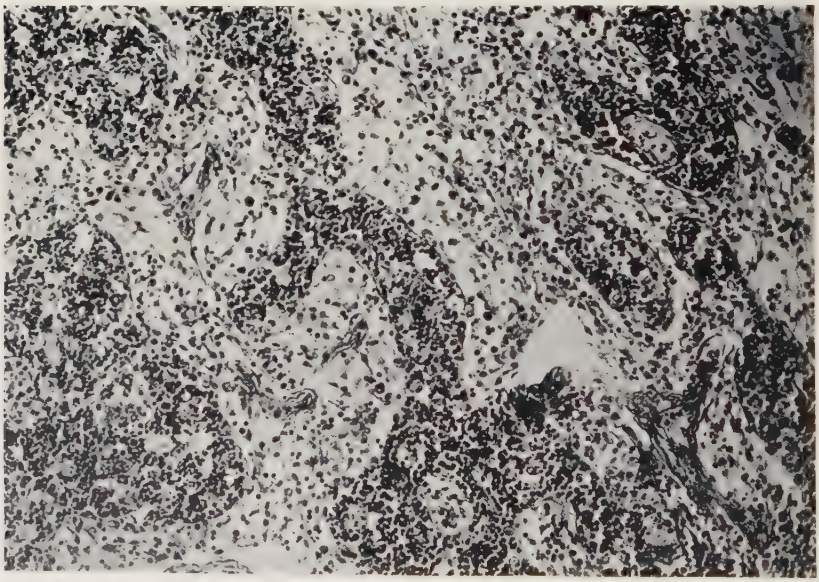

FIG. 21. PHOTOMICROGRAPH OF CERVICAL LYMPH-NODE FROM SAME REGION OF LITTER-MATE CONTROL FEMALE DOG (FIGS. 20 AND 22), SHOWING SLIGHTLY LESS DENSITY OF LYMPHOCYTES AND RELATIVE INCREASE OF RETICULAR ELEMENTS—AN APPROXIMATELY NORMAL PICTURE (× 80)

Lymphogenic organs. In the injected animals, the thymus and cervical lymph glands in particular, and lymph glands in other regions of the body (but not in the spleen), were enlarged. Microscopically there was a thinning out of lymphocytes in the organs, with an apparent relative increase in the amount of reticular tissue, which also had a loose edematous appearance. In the hypophysectomized animals, in which the organs are relatively smaller (this includes also the thymus), these gross conditions were more marked. Microscopically there was

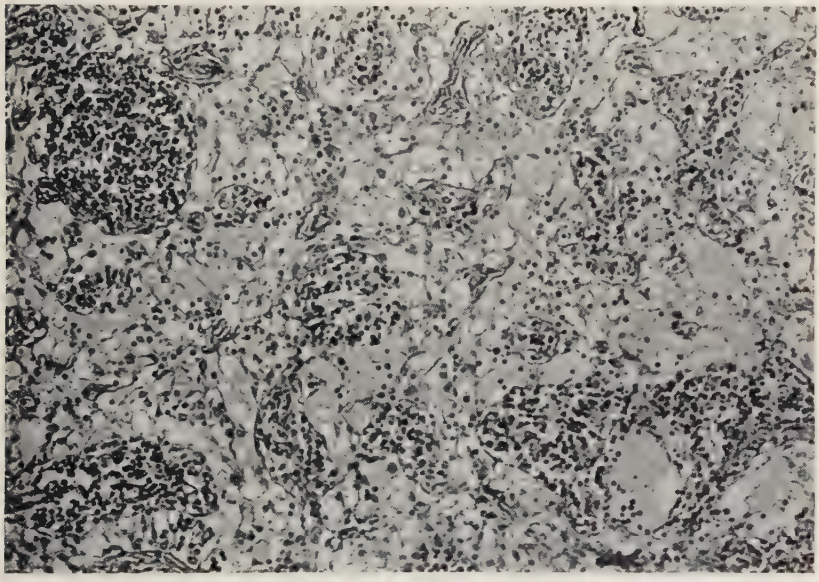

FIG. 22. PHOTOMICROGRAPH OF CERVICAL LYMPH-NODE FROM SAME REGION IN INJECTED LITTER-MATE FEMALE (FIGS. 20 AND 21), SHOWING RELATIVE THINNING OUT OF LYMPHOCYTES AND DISTINCT EDEMATOUS APPEARANCE OF RETICULAR TISSUE (× 80)

a relatively greater amount of lymphocytes, with a smaller amount of reticular tissue and none of the edematous appearance (*figs. 20, 21,* and *22*). In the controls, conditions were between the two types of treated animals in relative proportions of lymphocytes and reticular tissue, but there was no edematous state of the latter.

In the other visceral organs—*lungs, heart, intestinal tract,* etc.—there were no significant changes. The *male genitalia,* in both the first and last groups, revealed no marked microscopic differences.

Teeth. Microscopic study of the teeth was very disappointing. All of the animals received injections of trypan blue intra-peritoneally at intervals of a month. It was hoped that this would reveal lines of deposition in the dentin. The upper right cuspid tooth of each autopsied animal was fixed for ten days in neutral formalin, dehydrated, embedded in block in benzol damar, and ground in mid-sagittal section to 50 micra in thickness. This was mounted in balsam and carefully examined under the dissecting microscope. No evidence of deposition of trypan blue could be seen in any case (*figs. 9*, *10*, and *11*).

FIG. 23. FRONT VIEW OF ANTERIOR TEETH OF (LEFT) HYPOPHYSECTOMIZED MALE, *10*, SHOWING TUMBLING AND "FLARING" OF INCISORS AND CUSPIDS, AND OF (RIGHT) INJECTED LITTER-MATE MALE, *11*, SHOWING LARGER MAXILLAE, SPACING AND VERTICAL POSITION OF INCISORS AND CUSPIDS. SEE FIG. 24

Some of the remaining cuspids and other teeth were decalcified, sectioned, and stained; others ground to microscopic thinness, stained, and mounted for histological study. Sections stained with iron hematoxylin, hematoxylin and eosin, haemalum and congo red, and the trichromic stain of Masson, revealed no changes in the arrangement of enamel rods, apparent density of dentin, or variation in dentinal tubules. There was no variation in the calcified trabeculae of the surrounding bone. In no instance did the ameloblasts and odontoblasts show deviation in regularity of arrangement, or in relationship in size

or outline between cells and nuclei. Staining qualities of the hard structures, pulpal cells, and cells of the surrounding tissues, were not

FIG. 24. LATERAL VIEW OF HEADS OF SAME ANIMALS (FIG. 23)

Top: injected; showing frontal bulging, larger head and jaws, and vertical position of anterior teeth. *Bottom:* hypophysectomized; showing frontal flattening, smaller head and jaws, and "flaring" of anterior teeth due to diminished arch.

changed in either type of treated animal. Neither was their arrangement altered (*figs. 32–34, 38–40*). Many sections were stained with

Cowdry's mitochondrial stains, which, despite the ill effects of decalcification, revealed numerous cell inclusions, especially in the amelo-

FIG. 25. DRAWINGS OF CELLS FROM GROWING TEETH OF DOGS

a, ameloblasts from injected animal. *b*, odontoblasts from same animal. *c*, odontoblasts from control. *d*, ameloblasts from same animal. *e*, ameloblasts from hypophysectomized animal. *f*, odontoblasts from same animal. *g*, orientation sketch through developing root, showing (1) pulpal cells, (2) odontoblasts, (3) forming dentin, (4) forming enamel, (5) ameloblasts, (6 and 7) surrounding connective tissue cells. All stained for mitochondria after Cowdry. Inclusions may be seen clustering about the nuclei of the ameloblasts, but there were no differences for types of treatment.

blasts. For each type of animal these tended to clump around the nucleus, and showed no variation in accord with the types of handling (*fig. 25*).

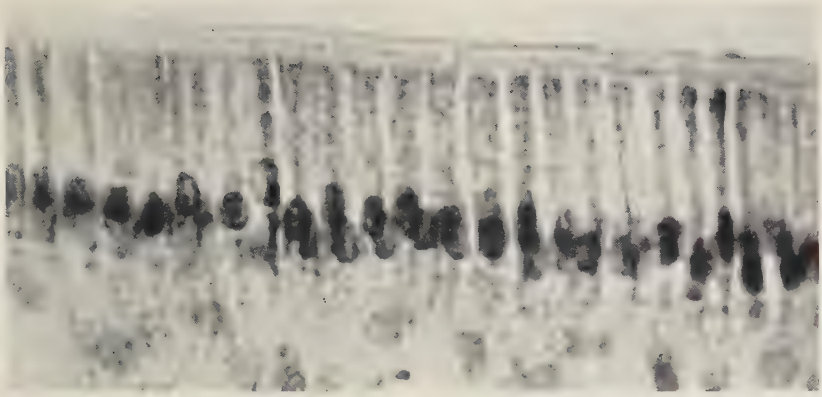

FIG. 26. PHOTOMICROGRAPH OF AMELOBLASTS FROM CONSTANTLY GROWING INCISOR OF CONTROL MOUSE DECALCIFIED H. AND E. SECTION. SEE FIGS. 27 AND 28 (× 1200)

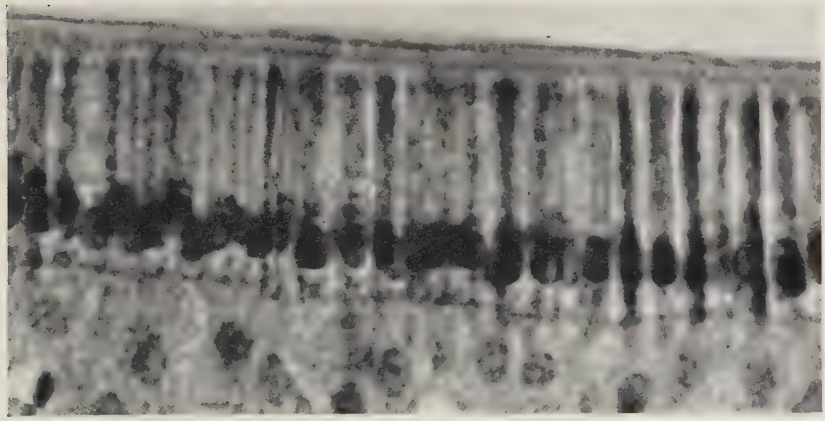

FIG. 27. PHOTOMICROGRAPH OF SIMILAR AREA AND PREPARATION FROM INJECTED MOUSE. LITTER-MATES: FIGS. 26 AND 28

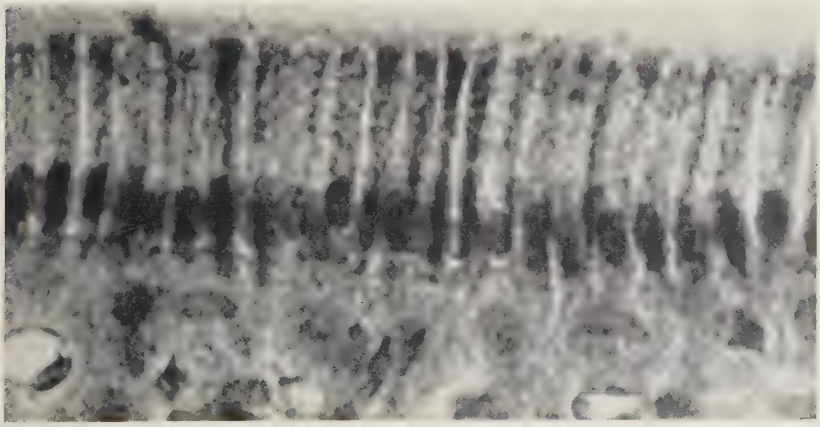

FIG. 28. PHOTOMICROGRAPH OF SIMILAR AREA AND PREPARATION FROM ANOTHER CONTROL MOUSE. NO DIFFERENCES IN ARRANGEMENT OR STAINING QUALITIES OF CELLS. LITTER-MATES: FIGS 26 AND 27

IV. CONCLUSIONS AND COMPARISONS OF RESULTS

Were it not possible to draw upon the work of earlier investigators, it still would be quite clear that the present work demonstrates the presence of a definite growth-promoting principle in the anterior lobe of the pituitary gland. In each instance, with each type of animal, a general increase in size and weight has been obtained by increasing the

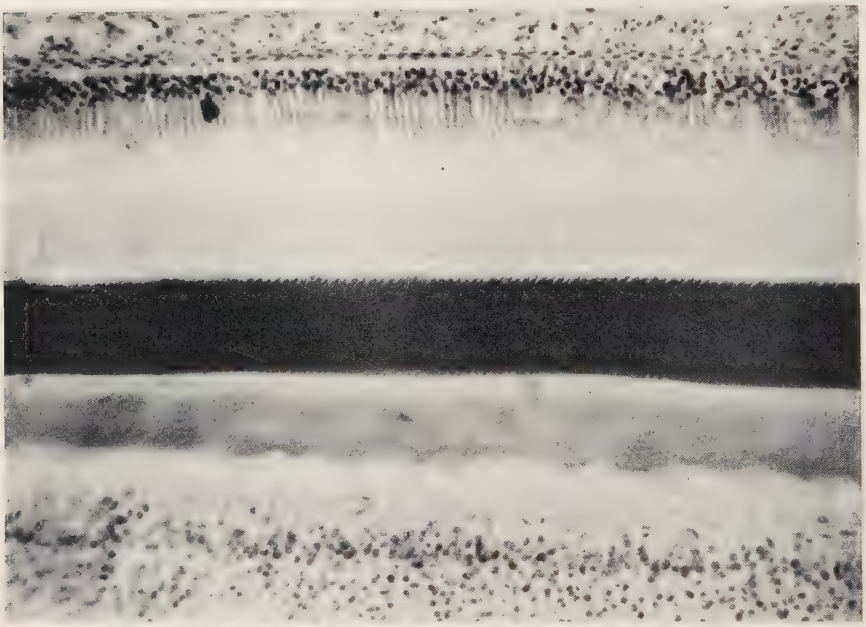

FIG. 29. PHOTOMICROGRAPH OF SECTION THROUGH WALL OF CONSTANTLY GROWING INCISOR OF INJECTED MOUSE, SHOWING ODONTOBLASTS ABOVE; FORMING DENTIN, FORMING ENAMEL (ON LABIAL ONLY), AMELOBLASTS BELOW. DECALCIFIED H. AND E. SECTION (× 600). SEE FIGS. 30 AND 31.

available quantity of the growth-promoting principle. In dogs, loss or ablation of the gland definitely inhibited growth in each case. It is true that these changes have not been parallel or identical in degree in all tissues or organs of a given animal. This does not, however, mitigate the fact that the difference was general, and exercised in the same direction for all of the animals on a given type of treatment. Stockard (73, 76), Donaldson (32), Jackson (88, 89), Scammon (150), and others,

have shown that growth is not a constantly increasing process in each of the tissues and organs, in the same degree. General growth goes on at a variable rate, in the same animal. Growth of the organs and tissues proceeds at different rates in the same individual over the same period. Such would seem to be the case with the growth stimulation caused by this principle from the anterior lobe. There was a general increase in size and growth of the animals injected, but this increase

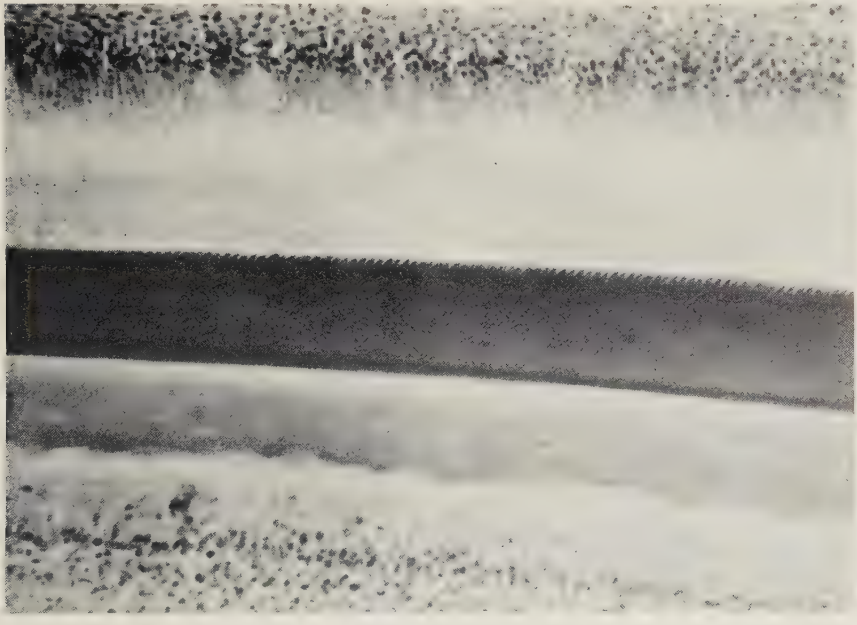

FIG. 30. PHOTOMICROGRAPH OF SIMILAR AREA AND PREPARATION FROM TOOTH OF LITTERMATE CONTROL. SEE FIGS. 29 AND 31

was more marked in certain organs than in others. The livers and internal female genitalia showed the greatest changes. The kidneys and lymphogenic organs were next in degree. The remaining organs, including the teeth and jaws, apparently shared only in the general increase—evidenced in the teeth by accelerated rate of eruption, but not by increase in size or by change in form; in the jaws, by increase in size as well.

Owing to microscopic conditions in the liver, kidneys, and lympho-

genic organs, possibly a part of their increase in size was due to a toxic effect of the experimental procedure. The nature of this toxic influence is difficult to determine. Possibly a toxic product arose during the preparation of the extract. But the fact that the effects seem to be very similar, almost regardless of the method used, does not point in this direction. Perhaps if cell metabolism is stimulated to a very

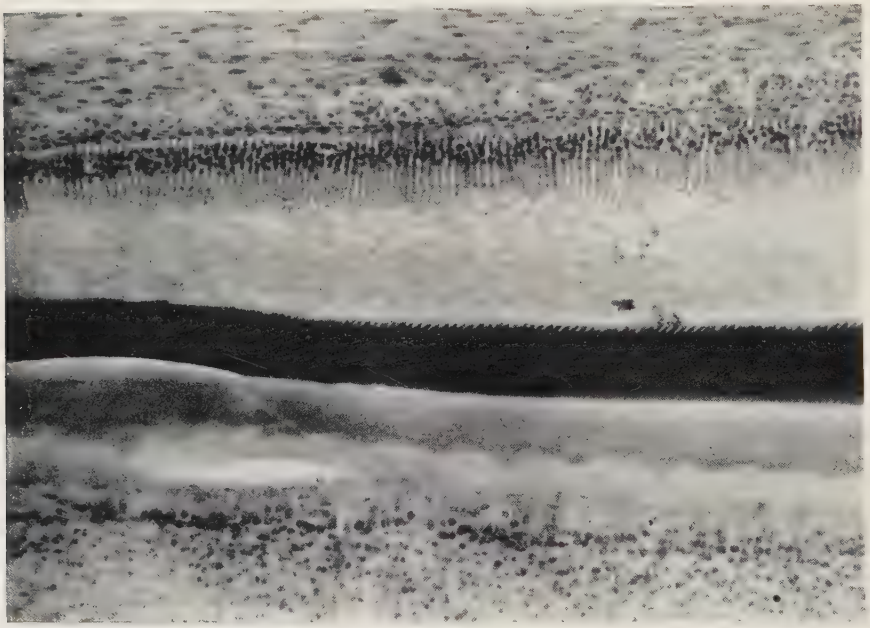

FIG. 31. PHOTOMICROGRAPH OF SIMILAR AREA AND PREPARATION FROM TOOTH OF INJECTED LITTER-MATE. THERE ARE NO DIFFERENCES IN SIZE, ARRANGEMENT, OR STAINING QUALITIES OF CELLS, OR HARD SUBSTANCES. SEE FIGS. 29 and 30

advanced degree, the by-products of this rapid change may be so abundant that they cannot be properly taken care of by the eliminative processes of the animal, with resultant toxic effect. In any event, it is obvious that, in dogs and mice, injection of the available extracts, with perhaps the exception of the "globulin" fraction (183)—which was not carefully studied in this instance—induces not only a marked general growth-effect, but also a definite toxic action that is most noticeable

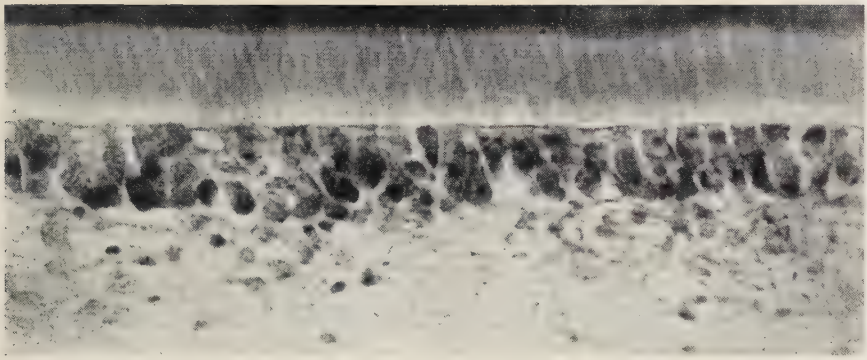

FIG. 32. PHOTOMICROGRAPH OF ODONTOBLASTS AND DENTIN FROM TOOTH OF HYPOPHYSECTOMIZED DOG. DECALCIFIED, MASSON-STAINED SECTION (× 800). SEE FIGS. 33 AND 34

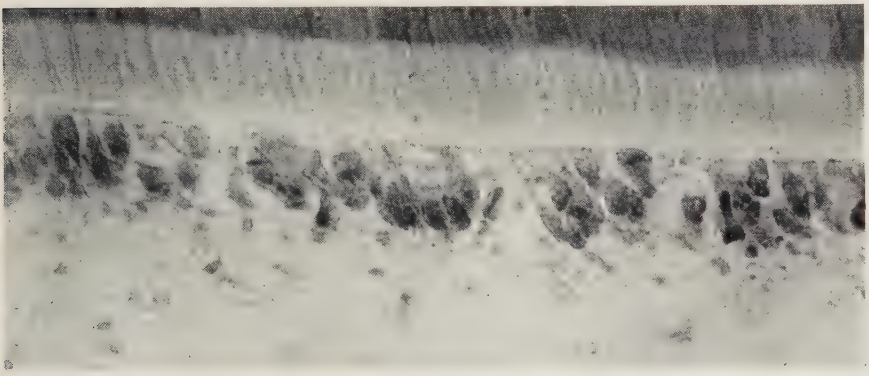

FIG. 33. PHOTOMICROGRAPH OF SIMILAR AREA AND PREPARATION FROM TOOTH OF LITTERMATE CONTROL. SEE FIGS. 32 AND 34

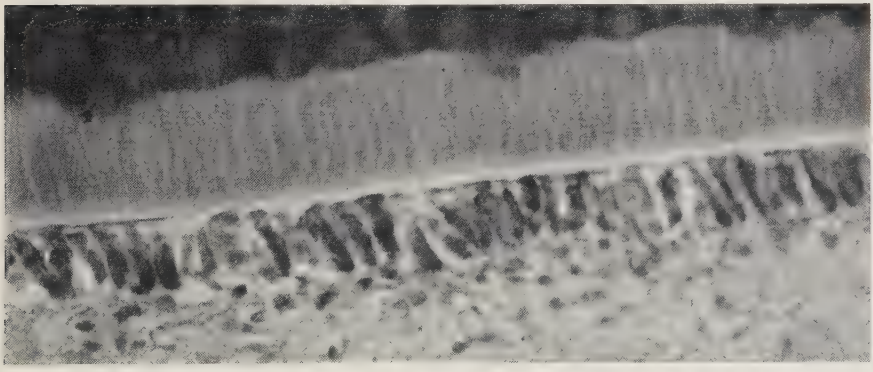

FIG. 34. PHOTOMICROGRAPH OF SIMILAR AREA AND PREPARATION FROM TOOTH OF INJECTED LITTER-MATE. THERE ARE NO DIFFERENCES COMPARED WITH FIGS. 32 AND 33

in the cells at the center of the lobules in the liver; slightly less so in the kidneys and lymphogenic organs.

The chemical changes noted in the mouse-injection experiments are particularly significant. The injections caused a continued high water content, with a somewhat complementary slower increase of fat content than in the controls. An analogous general effect was observed in the similarly treated dogs. Injections into mice caused a

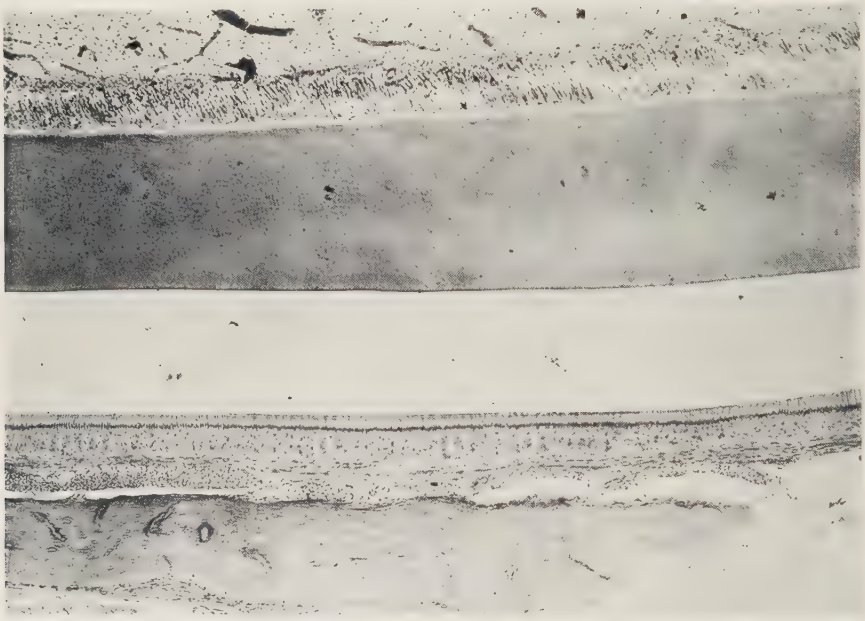

FIG. 35. PHOTOMICROGRAPH OF SECTION THROUGH WALL OF CONSTANTLY GROWING INCISOR OF INJECTED MOUSE. H. AND E. DECALCIFIED SECTION. (× 100). SEE FIGS. 36 AND 37

definite rise in the total ash content. Referring to Moulton's excellent study on tissue content, and paralleling it with Donaldson's on growth-rate at different periods, it appears likely that the chemical phenomena in the present studies, and usually found in somewhat younger animals, are parts of the picture of markedly stimulated growth, and were observed at the different ages because the repeated injections carried a normally early growth rate into a later period chronologically.

Apparently the rate of growth noted by Donaldson, in the "third phase," has been extended into a later time period. This should likewise account for the great increase in rate of eruption of the permanent dentition.

It is not impossible, but seems quite unlikely, that the recorded changes in water, fat, total ash, and nitrogen contents, are a part of the picture of toxicosis as revealed by the microscope. These changes, as

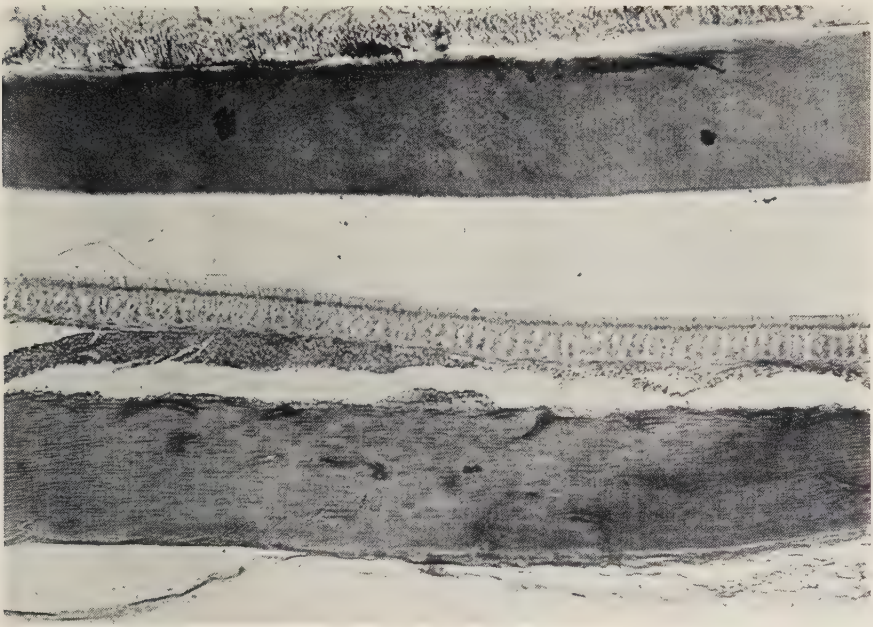

FIG. 36. PHOTOMICROGRAPH OF SIMILAR AREA AND PREPARATION, FROM CONTROL LITTERMATE. SEE FIGS. 35 AND 37

indicated by the curves plotted in each case (*figs. 3* and *4*), are nearly identical with those at different growth stages in Moulton's studies. It is more likely that the chemical differences represent simply differences in growth acceleration.

The changes in the internal female genitalia are very marked and the interpretation seems obvious. Ablation or excision of the hypophysis caused a retardation of the sex apparatus in all directions. This is logical in view of the fact that excision of the entire gland removes from

the animal the sex-stimulating principle necessary to normal sexual development. In the case of untreated controls, growth, both general and sexual, proceeded at a normal rate. The genitalia of these animals were much further advanced in each direction than those in the operated animals. In the genital organs of injected animals there was an increase in size and stroma compared with the control animals, but no notable difference in the sex-elements proper. Apparently the in-

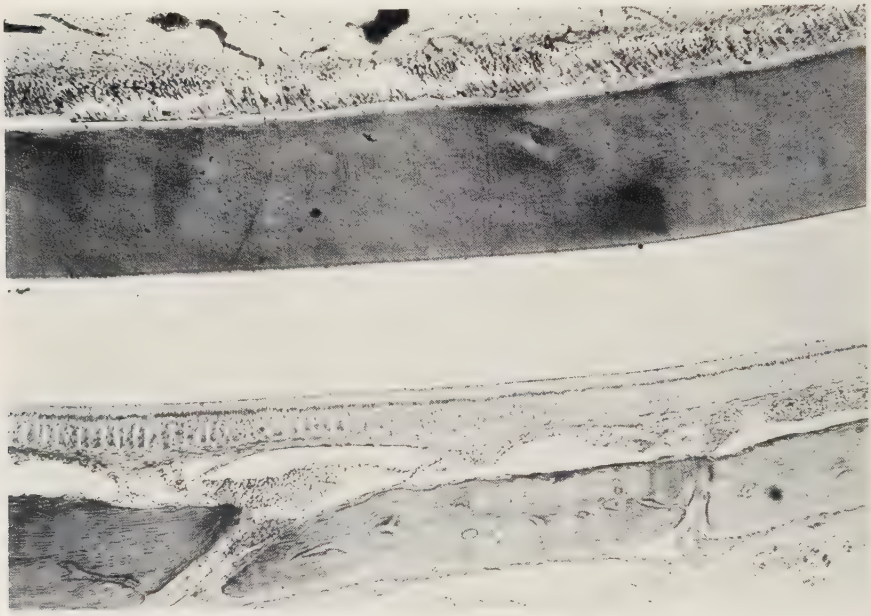

FIG. 37. PHOTOMICROGRAPH OF SIMILAR AREA AND PREPARATION, FROM CONTROL LITTERMATE. NO DIFFERENCES ARE NOTED. SEE FIGS. 35 AND 36

crease in size and stroma in this case was a phenomenon of general body growth, stimulated by the injection of the growth principle. But inasmuch as the sex-stimulating effect was only that of a normal animal, the picture of the sex-elements is not changed from that seen in the controls. The sex-elements in the males were not similarly affected, presumably because the male sex-mechanism is much less labile than that of the female.

The studies of Dott (33), Dott and Fraser (34), and others, have re-

vealed that there are fairly regular changes in the long bones in the various types of pituitary experimentation. They obtained definite changes in both hypo- and hyperpituitarism experimentally produced. X-ray studies on my animals were negative in this respect, except as to density and size of mandibulae and teeth in mice, and density of mandibulae in dogs. This observation does not necessarily contradict the finding of Dott and Fraser, but may indicate merely that many

FIG. 38. PHOTOMICROGRAPH OF SECTION THROUGH WALL OF GROWING TOOTH OF INJECTED DOG. DECALCIFIED H. AND E. SECTION. (× 80)
SEE FIGS. 39 AND 40

factors in addition to the purely personal one of interpretation, affect x-ray findings. The youth of my animals, even at the termination of the experiments, might have been a factor; faulty x-ray technic another. However, the lack of x-ray evidence is quite definite, except, as mentioned, in the mandibulae. These were denser in the injected animals of both types, but showed no difference as between hypophysectomized and control dogs. X-rays of the mandibulae of mice showed also increase in size of both teeth and bone. In the injected

dogs, increase in size and density of the bone was evident. Owing to the difference in maxillary size, the arrangement and spacing of the teeth were affected, but no change in either density or size could be seen.

In animals having diphyodont dentitions, their size and form appear to be laid down in accord with genetic principles entirely, and are consequently little if at all influenced experimentally. On the other hand,

FIG. 39. PHOTOMICROGRAPH OF SIMILAR AREA AND PREPARATION FROM TOOTH OF CONTROL LITTER-MATE. SEE FIGS. 38 AND 40

in rodents having constantly growing incisors, the size and form of these teeth might easily be altered by influences that modify the investing tissues. The results of the present type of experimentation would seem to bear this out. My earlier study (36, 37, 38), on causes of anomalies, laid down the importance of genetic factors in the pattern of the dentition. The studies of Putnam, Teel and Benedict, and those of Reichert (144 and 145), demonstrated, as does the present one, that variations in time of eruption of the teeth and size of the jaws

might be induced by pituitary experimentation. In their studies, the teeth and jaws were apparently not examined microscopically, but there was no gross evidence of change in form or structure of the teeth or investing tissues, except in the size of the jaws. So far as I am aware, no other similar studies have been pursued.

Wolbach and Howe (201–204), Höjer and Westin (79, 196, 197), Toverud (185), and others, have described very definite changes in the

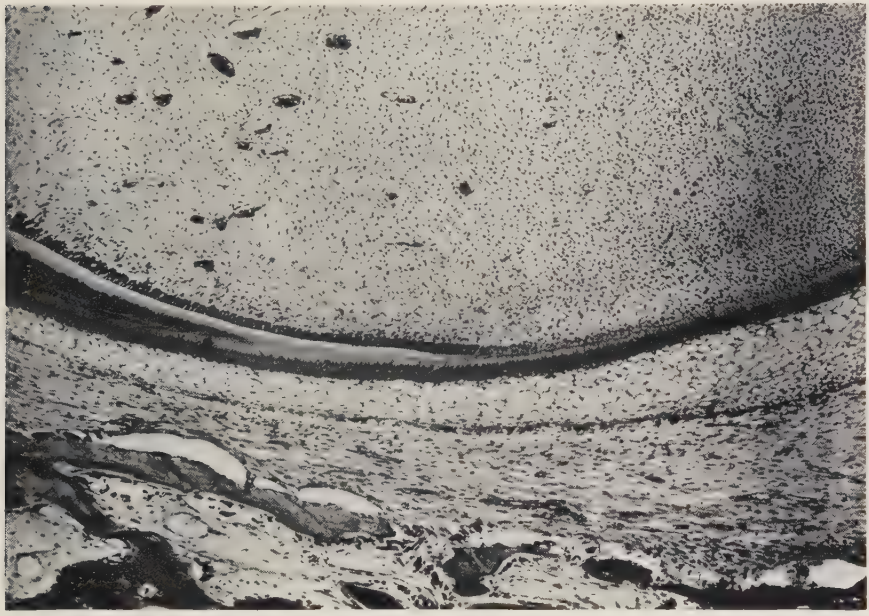

FIG. 40. PHOTOMICROGRAPH OF SIMILAR AREA AND PREPARATION FROM TOOTH OF HYPOPHYSECTOMIZED LITTER-MATE. THERE ARE NO DIFFERENCES.
SEE FIGS. 38 AND 39

pulpal cells in scurvy. The odontoblasts lose their regular palisade-like arrangement and osteodentin is formed in the pulp. The dentin may actually become resorbed. May Mellanby in particular (118–120), also Marshall (112), McCollum and co-workers (109), and others, have described changes in both the hard substances of the tooth and in the surrounding bone in rickets. Erdheim (45, 46), Toyofuku (186), Gies (64), and Hammett (71), have noted marked changes in density and

texture of the rodent incisors in parathyroid experiments. The preparations obtained from the present studies have been carefully checked against each of these, but no similar changes were noted.

It is felt, again, that this points to the eruption and development of the dentition as a phenomenon of general somatic growth. Likewise, the fact that the effect on the eruption of the permanent dentition, and their growth, is about parallel in time and rate with the growth changes in the body in general, demonstrates that this is true; and that the growth-fraction of the anterior lobe stimulates growth, i.e., development of the body in general, in time and in degree, and is not in any sense specific as to organs or tissues involved.

V. SUMMARY

The present study, initiated to determine effects of the growth-fraction of the anterior lobe of the pituitary body on the teeth and their investing tissues primarily, and on other tissues and organs secondarily, demonstrated the following facts:

1. The conclusions of numerous other workers that there is, in the anterior lobe of the hypophysis, a hormone or substance not necessary to life, but essential for normal or average growth and development, has been substantiated.

2. A part of rapid growth in animals is the late retention, in the tissues, of a larger percentage of water, a retardation in fat storage, and an increase in mineral concentration.

3. One effect of experimental hypophysis-stimulated growth is a definite toxic action on the tissues and organs most susceptible to such influences.

4. The growth-fraction of the hypophysis may accelerate the rate of eruption of the dentition; and, owing to increase in the size of the jaw, may also alter the position of the teeth; but, in the case of animals having a dyphyodont type of dentition, does not change the size or form of the teeth.

VI. AUTOPSY PROTOCOLS

Autopsy, dog 4. Hypophysectomized Oct. 17, 1929. Litter-mate of dogs 5 and 6

Female fox terrier, twenty-six weeks old. Superficial examination: underdeveloped and pot-bellied. Large amount of loose, baggy skin, espe-

cially around throat; sparse, irregularly distributed hair, scaly skin eruption over most of head and body. Weight, 8 lbs., 2 oz.; at beginning, 5 lbs., 14 oz. Legs short in proportion to size; ankles enlarged and edematous. External genitalia very infantile.

Body opened by midline incision extending from sterno-clavicular notch to symphysis pubis. Considerable amount subcutaneous, mediastinal, mesenteric fat. Pericardium quite fatty; no fluid in pericardial sac. Heart weighs 23.4 gm.; no gross abnormalities or changes. Right lung weighs 21 gm.; left, 22.2 gm. Same gross description applies to each: light pink, not collapsed or boggy, few small gray mottlings on surface—also seen on sectioned surface. Spleen weighs 8.3 gm.; no gross abnormalities. Pancreas weighs 12.7 gm.; grossly normal. Kidneys weigh 26.8 gm.; glistening and dark colored, firm; no gross variations in appearance of cortex, medulla, or pelvis; capsule strips with ease. Stomach and intestinal tract normal in general; clear pink mucous membranes; contain moderate amounts partially digested food and fecal matter, respectively. Liver moderate size, light red, glistening; homogeneous texture on section; weighs 118.4 gm. Uterus, tubes, ovaries: very small, thin, light-colored.

Skull severed; cleaned of adherent soft tissues for measurement. Tongue small but rough, gray, dense. Cervical lymph nodes and thymus small, firm. Salivary glands normal. All temporary teeth in situ except two lower incisors replaced by permanent. Cuspids, permanent, barely erupting anterior to temporary cuspids still in place and firmly-set in arch. Teeth generally crowded; "tumbled" appearance. Measurement from external occipital protuberance to tip of nasal spine, 10.5 cm.; from crest of "bow" of zygoma, to same point on opposite side, 6.5 cm.; from condyloid process of mandible to symphsis mentis, 7.5 cm., either side; from cuspid prominence to cuspid prominence, 2 cm. Cranial sutures in general closed but easily made out; not dense. Marked flattening in frontal region. Brain appears normal; no evidence of hemorrhage around sella turcica. Hypophysis severed cleanly; bony fault and sella turcica filled in with loose connective tissue of embryonic type (microscopically determined).

Autopsy, dog 5. Litter-mate of 4 and 6

Female fox terrier, twenty-six weeks old. Superficial examination: normally developed and nourished; no peculiarities of hairy distribution, proportion, or external genitalia. Weight, 8 lbs., 10 oz.; at beginning, 5 lbs., 2 oz. Body opened by usual incision; moderate amount subcutaneous, mediastinal, mesenteric fat. Pleural surfaces normal, smooth, glistening. Pericardium: no changes. Heart normal; weighs 33.3 gm.

Right lung weighs 38.3 gm.; left, 42.5 gm. Both lungs: grayish mottling and foaminess on collapse; cultures of bronchosepticus obtained. Spleen weighs 20.9 gm.; grossly normal. Liver large, firm, red, somewhat bloody; weighs 188.5 gm. Kidneys firm, dark red to purple, smooth, glistening; weighs 36.7 gm.; no gross abnormalities. Stomach and intestines: no gross abnormalities; moderately distended with partially digested food and fecal matter. Ovaries, uterus, and tubes: normal appearance, but larger than those in dog 4 (supra). Thymus, lymph-nodes, thyroid, salivary glands: all grossly normal.

Head separated; freed from adherent tissues. Tongue and oral mucous membranes normal. Four of permanent incisors completely in place. Permanent cuspids erupted far, and all but one of temporary cuspids shed. Three of four permanent first molars erupting. Cranial sutures still made out with ease, but well closed. Mandible cuts with greater difficulty than that of dog 4. Dura, brain, sella turcica, hypophysis: no changes.

Autopsy, dog 6. Daily injections of Evans extract begun Oct. 24, 1929. Litter-mate of dogs 4 and 5

Female fox terrier, twenty-six weeks old. Superficial examination: large, tall, well developed, well nourished; sleek thick hair-coat; advanced development of external genitalia. Weight, 10 lbs., 2 oz.; at beginning, 4 lbs. Toe-nails extremely long and hard. Body opened as usual. Zyphoid process: much calcification. Almost no subcutaneous, mediastinal, or mesenteric fat, but large amount of thymic tissue. Pericardium and heart: grossly normal; latter weighs 62.7 gm. Right lung weighs 84.2 gm.; left, 63.2 gm.; both rather dark and dense; yield culture of bronchosepticus. Liver large, brown, firm; weighs 268.9 gm.; brown sectioned surface—distinct lobules. Pancreas, grossly normal; weighs 15.6 gm. Spleen: no abnormalities; weighs 17.4 gm. Kidneys weigh 46.5 gm.; red, apparent hemorrhagic area around pyramids not seen in other animals of group. Ovaries, uterus, tubes: grossly much larger than in other animals of group. Peritoneum: smooth, glistening; no gross evidence of pathological change from numerous intraperitoneal injections.

Head severed; soft tissues dissected away. Cervical lymph glands markedly enlarged. Thyroid, salivary glands: no gross change. Tongue greatly enlarged, ridged, rough; lacks clear normal outlines. Cephalic measurements, 12.5 × 8.5 cm. Cranial sutures only barely visible. Definite "bulging" in frontal region, distinctly suggestive of clinical condition in acromegaly. Mandible 8.5 cm. in length. Distance between cuspid eminences, 2.8 cm. Permanent cuspids all completely erupted; upper two

temporary not cast off. All of first permanent molars in place; second ones erupting. Entire dentition much further advanced than in control dog 5. Mandible: massive, teeth spaced; sections with great difficulty. Dura, brain, sella, hypophysis: no abnormality.

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